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Molecular Genetics, Microbiology, and Prehistory

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Also relates to Avery

Since the literature of molecular genetics has been growing explosively, it is small wonder that those who were not present at the creation of the field may have a cloudy picture of its origins: events of only five years ago are already ancient history. But a recent reprinting of the autobiography of Emil Fischer has brought to light an even earlier prehistory, in which a remarkable speculation anticipated the possibility of genetic engineering. To appreciate this contribution we must first consider the obstacles that so long delayed the union of biochemistry and genetics.

The Role of Bacterial Genetics

Everyone knows, of course, that the discovery of the double helix by Watson and Crick in 1953 gave birth to molecular genetics. But the field had its conception – the zygote that united its previously disparate elements – in the chemical identification of the pneumococcal transforming principle by Avery, MacLeod and McCarty in 1944. Moreover, this discovery not only established DNA as the material of the gene: it also launched the field of bacterial genetics. For genetic studies, until the recent identification of gene sequences, had depended on the recombination of different alleles from different parents. Transformation first demonstrated that this process occurs in bacteria, and it quickly led to Lederberg's recognition of two additional mechanisms, conjugation and transduction.

The importance of bacterial genetics for the development of molecular genetics cannot be overemphasized. The work of Beadle and Tatum with auxotrophic mutants of the mold Neurospora not only provided a novel set of genetic markers in a single-celled organism; it also indicated that each gene forms a corresponding enzyme; and similar mutants of bacteria soon provided a wide variety of phenotypes that invited similar biochemical correlations. Moreover, while recognition of the double-helical structure of DNA was based on biochemistry and X-ray crystallography, exploration of the implications of that structure benefited from a radical advance provided by bacterial genetics: while classical genetics could only count the various phenotypes in a limited population of progeny, appropriate selective media for bacteria, or selective hosts for their viruses, could be used to isolate even very rare mutants or recombinants from an enormous population. The resolving power of genetic mapping was thus suddenly refined by many orders of magnitude: mutations and recombinations, previously localized only in terms of genes, could now be localized in terms of individual nucleotides.

Possible Reasons for the Neglect of the Avery Discovery

The Avery discovery was truly revolutionary, not only because of its intrinsic significance, but because the answer was so unexpected. Before then it was generally assumed that only proteins could provide the complexity required for the gene, and so the DNA in the chromosomes was presumably providing some kind of scaffolding. Yet Avery and his colleagues did not receive a Nobel Prize, though he lived for 11 years after announcing the role of DNA. Neither was a Nobel Prize awarded for the development of the fine-structure genetics by Benzer, Yanofsky and Brenner which made it possible to correlate specific changes in DNA sequence with changes in protein sequence and with altered function in the cell. It may be hard to appreciate today that this discovery – that crossing-over can occur between any adjacent bases, rather than at special sites between genes – had the two elements of a great discovery: surprise, as well as broad significance. Perhaps the problem here was that fine-structure mapping, and the collinearity of DNA and proteins, so quickly became taken for granted as a foundation on which so many built.

Since the Nobel Committee has on the whole shown excellent judgment, except in the area of medical therapy (for example, Finsen's prize for allegedly curing skin tuberculosis with ultraviolet irradiation, and Munoz's for prefrontal lobotomy), we must wonder why they missed Avery. Indeed, this was clearly one of their most egregious errors (though some would consider bypassing Freud's impact on literature, if not on medicine and psychology, an even greater omission). Here I would like to suggest several reasons for the slow recognition of Avery and his colleagues.

(1) A small part of the responsibility rests on Avery himself, for while his unique style was something to admire, it was not ideally designed to draw attention to such a revolutionary discovery. Indeed, it was antithetical to the inimitable highly competitive, impatient style set by the subsequent leaders in molecular genetics, continually shifting the direction of research as new peaks were revealed for conquest. Avery instead devoted his entire lifetime to the patient study, with few collaborators and little sense of competition, of factors affecting the virulence of one organism, the pneumococcus – the major cause of death in the developed parts of the world at the time when he began.

What is even more relevant was his style of publication, which would clearly be very difficult to emulate in today's era of intense competition and constant pressure to justify renewal of grants. My teacher, Dubos, told me that Avery would explore a problem at length and finally, when he had the answer, would not publish these data but would perform the 'protocol experiment', with the precise number of points and controls needed to document his conclusions. Moreover, he would then put the paper in the drawer for a few months in order to be able better to polish it before publication – and the yield was at most two to three papers per year. In addition, he was opposed to short notes: it did not escape his attention that the DNA discovery had deep genetic implications, which he expressed most tentatively; but he published it only as a full paper in the *Journal of Experimental Medicine*, without trying to draw attention to its general significance in such a journal as *Nature*. A few years later, visiting what was then the world center of research on biochemical genetics, Beadle's department at CalTech, I found that its

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library, understandably, did not carry the *Journal of Experimental Medicine*.

(2) The cleft between genetics and bacteriology was an even deeper problem. No chromosome had yet been seen in bacteria. Moreover, genetic adaptation in these organisms was generally confused with physiological adaptation, because overnight outgrowth of a variant seemed too rapid for a Darwinian process (until the power of rapid exponential growth and sharp selection was later understood). Accordingly, the study of bacterial variation was not yet linked to genetic concepts: inheritance in bacteria was generally ascribed to a vague plasticity, in the absence of evidence for the linked genes found in higher organisms.

The 'medical' basis of Avery's discovery further deepened the cleft. At that time bacterial capsules were studied only as virulence factors, and not as products of metabolic pathways; hence there was no basis for interpreting their formation in terms of specific enzymes, which might have linked transformation to the biochemical genetics recently initiated by Beadle and Tatum. Hotchkiss's subsequent transformation of more conventional biochemical traits eventually eliminated this barrier, but transformation probably long continued to seem strange to most biochemists.

The pneumococcus was then also very strange to most Drosophila and maize geneticists; it was not obvious, as it is now, that the evolutionary continuity of bacteria and eukaryotes implies shared common basic features of inheritance. To be sure, two insightful geneticists, George Beadle and Herman Muller, quickly pointed out in reviews the potential significance of pneumococcal transformation for their field; but their impact does not seem to have been large.

(3) A more important reason for the delayed general appreciation of Avery was the unexpected nature of his conclusion, which inevitably generated skepticism. Probably the greatest source of this skepticism, as candidly revealed in Maclyn McCarty's modest history of the discovery,[1] was a colleague at the Rockefeller Institute, Alfred Mirsky, who had been concentrating on chromosomes and nucleic acid for many years. He clearly did not enjoy being upstaged by these three medical microbiologists, all without a background in genetics, and self-taught in their biochemistry. What is more, Mirsky was an urbane, widely traveled man, and he gave many seminars emphasizing that the activity of even the purest DNA preparations might be due to contaminating protein. (It took painstaking experiments by Hotchkiss to show that the traces of amino acids, always present in hydrolysates of the DNA, were products of breakdown of purines.) Avery, who never went to meetings or traveled to give seminars, simply waited for Nature to settle the controversy. This it did – but too late for the Nobel Committee.

(4) An additional factor was that genetics was then a very specialized field, and a member of the Nobel Committee later explained to me that it was hardly represented at all in Sweden. And despite the aura of immortality surrounding the Nobel Prizes, the finite interests and background of the mortals composing the awarding committees obviously affect the selection.

(5) The role of phage geneticists in the skeptical reaction has elicited a good deal of controversy. This brilliant group had set out to study phage because this simplest of all organisms seemed most likely to reveal the nature of the gene. Since theirs was a much more logical approach than the serendipitous one that worked for Avery, it is understandable that they could easily find grounds for doubt about the genetic significance of his discovery. Only in 1952 did the phage group place its imprimatur on Avery's conclusion, when Hershey and Chase showed that labeled DNA of an infecting phage entered the host cell while the differentially labeled protein remained outside. But while this pioneer phage experiment was a most important one, it was not nearly as clean as the Avery experiment: the entry of the DNA was accompanied by about 20% of the phage protein, while the purified pneumococcal factor contained no detectable protein, and the activity was destroyed by DNase but not by protease.

(6) As has often been pointed out, the Watson–Crick discovery of DNA structure had a tremendous and immediate impact because its functional implications – for both gene replication and mutation – were obvious, while the Avery discovery implied only that DNA was important. But that does not explain why so few people took up that provocative lead in the next few years. This was a classical case of conservatism in scientific fashions, perhaps partly explained by the other features of the Avery discovery that I have just described.

Emil Fischer's Speculation about Genetic Engineering

These ruminations on early history cover ground that will be familiar to many readers. But I would like in addition to call attention to a less familiar speculation by Emil Fischer in 1914, foreshadowing genetic engineering. Fischer was a giant who gave us much of what we know about the organic chemistry of sugars, peptides, lipids and nucleic acids. Springer Verlag has just republished his posthumous autobiography, *Aus meinem Leben*, with a scholarly prologue by Bernhard Witkop of the NIH. Witkop notes the following passage, where Fischer[2] was discussing the methylated purines that he had been synthesizing:

> With the synthetic approaches to this group we now are capable of obtaining numerous compounds that resemble, more or less, natural nucleic acids. How will they affect various living organisms? Will they be rejected or metabolized or will they participate in the construction of the cell nucleus? Only the experiment will give us the answer. I am bold enough to hope that, given the right conditions, the latter may happen and that artificial nucleic acids may be assimilated without degradation of the molecule. Such incorporation should lead to profound changes of the organism, resembling perhaps permanent changes or mutations as they have been observed before in nature.

Of course, this prophetic speculation, not yet ripe for testing, fell by the wayside and was not a contribution toward the development of genetic engineering. Nevertheless, it reminds us that highly intelligent individuals, deeply immersed in an area of science, can offer judgments that are remarkably far-sighted.

REFERENCES

1 McCarty, M. (1985). *The Transforming Principle*. Norton.
2 Fischer, E. (1914). *Berichte* **47**, 3196.

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